

WHAT IS CLAIMED IS:

1. A pair of primers for specifically amplifying an hsp 65 (Heat Shock Protein 65) gene fragment of mycobacterial species comprising the
5 nucleotide sequences as shown in SEQ ID NO: 55 and SEQ ID NO: 56.
2. A polynucleotide of an hsp 65 gene fragment of mycobacterial species wherein the fragment is amplified by using a pair of primers specifically amplifying the hsp 65 gene fragment of mycobacterial species
10 comprising the nucleotide sequences as shown in SEQ ID NO: 55 and SEQ ID NO: 56.
3. A polynucleotide selected from the group consisting of polynucleotides as shown in SEQ ID NO: 1 to SEQ ID NO: 54, and
15 polynucleotides complementary thereto.
4. A polynucleotide set for the detection or identification of mycobacterial species wherein the set comprises at least an hsp 65 gene fragment selected from the group consisting of the polynucleotides as shown
20 in SEQ ID NO: 1 to SEQ ID NO: 54 and polynucleotides complementary thereto.

5. A method for the identification of mycobacterial species comprising the steps of:

(1) amplifying an hsp 65 gene fragment of mycobacterial species of interest with primers for specifically amplifying the hsp 65 gene fragment;

5 (2) analyzing a nucleotide sequence of the amplified hsp 65 gene fragment; and

(3) comparing the nucleotide sequence of the amplified hsp 65 gene fragment obtained in step (2) with a 604-bp hsp 65 gene fragment of a reference mycobacterial species.

10

6. The method of claim 5, wherein the primers comprise the polynucleotides as shown in SEQ ID NO: 55 and SEQ ID NO: 56.

7. The method of claim 5, wherein step (3) of comparing the nucleotide
15 sequence of the mycobacterial species of interest with that of a reference mycobacterial species is performed by multi-aligning the nucleotide sequence of the 604-bp hsp 65 gene fragment of the mycobacterial species of interest with a polynucleotide set of claim 4 to infer a phylogenetic tree.

20 8. A method for the identification of mycobacterial species comprising the steps of:

(1) amplifying an hsp 65 gene fragment of mycobacterial species with primers of claim 1; and

(2) analyzing the amplified fragment according to the RFLP (Restriction Fragment Length Polymorphism) analysis method using a restriction enzyme recognition site in the amplified fragment.

5 9. The method of claim 8, wherein the restriction enzyme is Xho I.

10. The method of claim 9 comprising the step of treating the amplified hsp 65 gene fragment with Xho I to produce restriction fragment(s), and analyzing the restriction fragment(s) according to an RFLP analysis method
10 to differentiate TB complex (*Mycobacterium tuberculosis* complex) and MOTT (*Mycobacteria* other than *Mycobacterium tuberculosis*).

11. The method of claim 10, wherein the restriction fragments are 391-bp, 150-bp, and 103-bp fragments to identify the TB complex.

15

12. The method of claim 10, wherein the 644-bp hsp 65 gene fragment is not cleaved by a restriction enzyme to identify fast-growing mycobacteria of MOTT.

20 13. The method of claim 10, wherein the restriction fragments are 391-bp, 169-bp, and 48-bp to identify a mycobacterial species selected from the group consisting of *M. avium*, *M. intracellulare*, *M. celatum*, *M. shimoidei*, and *M. szulgai*.

14. The method of claim 10, wherein the restriction fragments are 391-bp and 253-bp to identify a mycobacterial species selected from the group consisting of *M. gastri*, *M. genavense*, *M. gordonae*, *M. haemophilum*, *M. kansasii*, *M. malmoense*, *M. marinum*, *M. scrofulaceum*, *M. simiae*, and *M.*
5 *ulcerans*.

15. A kit for the differentiation or diagnosis of TB complex and MOTT comprising a pair of primers of claim 1 and Xho I, wherein the mycobacterial species is differentiated or diagnosed based on the size of restriction
10 fragment(s) which is obtained by amplifying an hsp 65 gene fragment of mycobacterial species in a sample with the primers to produce an amplified fragment and analyzing the amplified fragment according to an RFLP analysis method.

15 16. A method for the identification of a mycobacterial species comprising the steps of:

(1) amplifying an hsp 65 gene fragment of a mycobacterial species of interest with primers for specifically amplifying an hsp65 gene of mycobacteria; and

20 (2) hybridizing the amplified hsp65 gene fragment with a probe set comprising at least a probe selected from the group consisting of polynucleotides as shown in SEQ ID NO: 1 to SEQ ID NO: 54 and polynucleotides complementary thereto.